with experimental data in all scenarios. Of particular biological significance, our model predicts a threshold σ_s value below which the NC binding affinities reduce drastically and drop lower than that of single anti-ICAM-1 molecule to ICAM-1; our results reveal that this is due to a change in the multivalency (or number of bonds formed per NC). The trend and threshold values are exactly recovered by the *in vivo* measurements of the endothelium targeting of NCs in the pulmonary vascular in mice [Liu *et al*. *PNAS* 107: 16530-16535 (2010)]. Increasing the shear flow rate enhances the NC binding affinities till a threshold value is reached; this quantitatively agrees with existing experiments and a novel mechanism is revealed based on our model results. On this basis, our computational protocol represents a quantitative and predictive approach for model driven design and optimization of functionalized nanocarriers in targeted vascular drug delivery.

This work is supported by NIH Grants: R01-EB006818 (D.M.E.), R01-HL087036 (V.R.M.), and NSF Grant: CBET-0853389 (R.R.).

Imaging and Optical Microscopy III

3328-Pos Board B433

Characterization of Binding Affinity and Epitope Dynamics of Anti-HIV-1 Antibodies

Meron Mengistu, Krishanu Ray, Joseph R. Lakowicz, Anthony L. DeVico.

A preventive vaccine is potentially the most effective way to control the HIV pandemic. Such a vaccine needs to successfully harness humoral immunity and produce cross-reactive anti-envelope antibodies that mediate direct virus neutralization and/or Fc receptor-dependent killing. For these antibodies to carry out their functions in clearing HIV infection, they must bind the virus and prevent it from infecting target CD4+ cells. The capacity of an antibody to do this is dependent on the timing, duration and extent of cognate epitope exposure before and during the attachment and entry processes. The goal of this study was (i) to quantify antibody binding to HIV, and (ii) to characterize when and for how long antibody epitopes are exposed before and during virus-cell fusion. We studied the binding properties and epitope dynamics of antibodies against HIV envelope gp120 [b12 (CD4 binding site), 2G12 (carbohydrate clusters), A32 (C1, C4, & C5 domains)], CD4-induced epitope of gp120 (17b & 19e), and the membrane-proximal external region (MPER) of gp41 (4E10). To directly quantify antibody binding to virus in solution, we developed a fluorescence correlation spectroscopy (FCS) methodology that uses fluctuations in fluorescent signals to measure diffusion and reaction kinetics of fluorescently-labeled anti-envelope Mabs as they attach to HIV-1-JRFL, HIV-1-Bal, and HIV-1-NL4-3 pseudoviruses and infectious molecular clones. We have also developed methods to visualize the temporal appearance and disappearance of cognate epitopes during virus-cell fusion using immunofluorescence and live-cell imaging techniques. In this case, viral particles were labeled with a novel SNAP-tag technology that permits tracking of particles during different stages of fusion with CD4+ target cells, and concurrent imaging of epitopes that become exposed on the HIV envelope.

3329-Pos Board B434

An Improved Method for Studying Single Proteins Trapped in Lipid Vesicles

Claudiu C. Gradinaru.

We report on an improved method to encapsulate proteins inside surface-tethered liposomes in order to reduce or eliminate environmental interference for single-molecule investigations. The liposomes are large enough for the molecule to experience free diffusion, but sufficiently small so that the molecule appears effectively immobile for fluorescence imaging. Various single-molecule fluorescence experiments were performed to fully characterize this anchoring method relative to direct immobilization via biotin-streptavidin linkers. Multidimensional histograms of intensity, polarization and lifetime reveal that molecules trapped in liposomes display a narrow distribution around a single peak, while the molecules directly immobilized on surface show highly dispersed values for all parameters. For instance, when TMR-labelled molecules were immobilized directly on surface, we recorded large intensity fluctuations (6.30 ± 4.91 emission states/molecule), whereas the fluctuations were much smaller for the vesicle-trapped molecules (1.37 ± 0.71 emission states/molecule). During sample preparation, by hydrating the lipid film at low volumes, high encapsulation efficiencies can be achieved with ~10 times less biological material than previous protocols. By measuring directly the vesicle size distribution, we found no significant advantage for using freeze-thaw cycles during vesicle preparation. On the contrary, the temperature jump can induce irreversible damage of fluorophores and it reduces significantly the functionality of proteins, as demonstrated on single-molecule binding experiments involving a peptidic inhibitor for the oncogenic protein STAT3. Our improved and biologically gentle molecule encapsulation protocol has a great potential for widespread applications in single-molecule fluorescence spectroscopy.

3330-Pos Board B435

In Vivo Structure of the E. coli FtsZ-Ring Revealed by Photoactivated Localization Microscopy (PALM)

Guo Fu, Tao Huang, Jackson Buss, **Carla Coltharp**, Zach Hensel, Jie Xiao.

The FtsZ protein, a tubulin-like GTPase, plays a pivotal role in prokaryotic cell division. In vivo it localizes to the midcell and assembles into a ring-like structure-the Z-ring. The Z-ring serves as an essential scaffold to recruit all other division proteins and generates contractile force for cytokinesis, but its supramolecular structure remains unknown. Electron microscopy (EM) has been unsuccessful in detecting the Z-ring due to the dense cytoplasm of bacterial cells, and conventional fluorescence light microscopy (FLM) has only provided images with limited spatial resolution (200-300 nm) due to the diffraction of light. Hence, given the small sizes of bacteria cells, identifying the in vivo structure of the Z-ring presents a substantial challenge. Here, we used photoactivated localization microscopy (PALM), a single molecule-based super-resolution imaging technique, to characterize the in vivo structure of the Z-ring in E. coli. We achieved a spatial resolution of ,35 nm and discovered that in addition to the expected ring-like conformation, the Z-ring of E. coli adopts a novel compressed helical conformation with variable helical length and pitch. We measured the thickness of the Z-ring to be ,110 nm and the packing density of FtsZ molecules inside the Z-ring to be greater than what is expected for a single-layered flat ribbon configuration. Our results strongly suggest that the Z-ring is composed of a loose bundle of FtsZ protofilaments that randomly overlap with each other in both longitudinal and radial directions of the cell. Our results provide significant insight into the spatial organization of the Z-ring and open the door for further investigations of structure-function relationships and cell cycle-dependent regulation of the Z-ring.

3331-Pos Board B436

Assessing the Cellular Uptake Pathway for Poly-Lysine Analogues using Triplet Lifetime Imaging

Matthias Geissbuehler, Zuzana Kadlecova, Iwan Märki, Mattia Matasci, Dimitri Van De Ville, Harm-Anton Klok, Theo Lasser.

Research on synthetic delivery vectors is of major interest for cell imaging and manipulation, as they allow an efficient transfer of nucleic acids, therapeutic proteins or small drugs into the cells. We have developed a library of L-lysine analogues that allow for highly efficient gene delivery with low cytotoxicity. However little is known on the exact mechanism of uptake and the final intracellular destination of the synthetic carriers. Therefore we have developed a novel optical technique based on a modulated excitation allowing for intracellular imaging of the triplet-lifetime and -yield of fluorophores attached to the delivery vector. Both these parameters are highly dependant on the intracellular environment thus provide insight into the subcellular localization of the labelled carrier. The method combines high temporal and spatial resolution and is compatible with a multiplicity of fluorophores.

We performed series of model experiments to compare the triplet lifetime and triplet yield behaviour during the natural uptake mechanism to a series of controlled conditions. The latter include microinjection of fluorescently labelled carriers directly into the cytoplasm and cell nucleus as well as in vitro measurements under conditions mimicking physiological, acidic, or DNA rich environments. To validate our technique the results from the triplet imaging were compared with two complementary methods: carrier localization by subcellular fractionation and confocal laser scanning microscopy.

— Reference —

Geissbuehler et al. Triplet imaging of Oxygen consumption during the contraction of a single smooth muscle cell (A7r5). Biophysical Journal (2010) vol. 98 (2) pp. 339-349

3332-Pos Board B437

Correlative EFTEM, Stem and Fluorescence Microscopy as a Tool for Chromatin Biology

Maria A. Aronova, Alioscka A. Sousa, Guofeng Zhang, Michael J. Kruhlak, Elissa P. Lei, Richard D. Leapman.

In eukaryotes, the highly coordinated gene expressions require sophisticated levels of regulation. One such mechanism regulates the spatial and temporal organization of genes and their associated sequences in higher-order chromatin domains. Chromatin insulators, specific gene regulatory assemblies, form large nucleoprotein complexes known as insulator bodies and are thought to influence the organization of higher-order chromatin domains. In order to test current models of insulator function and provide ultrastructural information about these chromatin based domains, we use a correlative microscopy approach based on light microscopy and electron microscopy operating in the mode of energy filtered transmission electron microscopy (EFTEM) and scanning transmission electron microscopy (STEM).

We explore the ultrastructure of the well-studied *Drosophilamelanogaster-gypsy* chromatin insulator by immunolabeling a key insulator protein CP190 using a fluoronanogold conjugated antibody probe. In our correlative method, fluorescent imaging is initially performed to identify nuclei that contain insulator bodies, which are rare within thin sections. A comparison of low-magnification EM image of a whole cell with the corresponding fluorescent image reveals the approximate location of the structure of interest. The fluorescence signal observed by light microscope guarantees the presence of the conjugated nanogold, which can be visualized using STEM, and used to locate precisely the labeled CP190 proteins. EFTEM is then performed to image the distribution of nitrogen and phosphorus and thus map the distributions of protein and nucleic acid. It is evident from these two elemental maps that the insulator body contains an abundance of protein but a small quantity of nucleic acid. Even though dense chromatin surrounds the insulator body, it is difficult to determine whether the low levels of phosphorus within the insulator body structures correspond to DNA or RNA, which requires further investigation.

3333-Pos Board B438

Dominant Vinculin Binding Angle in Podosomes Revealed by High Resolution Optical Microscopy

Susan Cox, Marie Walde, James Monypenny, Rainer Heintzmann, Gareth Jones.

Podosomes are dynamic actin-rich cell-matrix adhesion sites of migrating and invasive cells such as macrophages and osteoclasts, and are receiving increasing attention due to their possible involvement in physiological events such as monocyte extravasation and tissue transmigration, as well as pathological conditions such as atherosclerosis, osteoporosis and cancer metastasis. These structures were examined using three different fluorescence microscopy techniques which provide resolution below the diffraction limit: structured illumination microscopy, stimulated emission depletion microscopy and stochastic optical reconstruction microscopy have been used. In high resolution images, it is clearly visible that each podosome consists of an actin core surrounded by a protein-enriched ring, supporting the existing podosome model. However, these rings are polygonal structures rather than smooth circles. An analysis of the binding angles at corners reveals vinculin to have a dominant binding angle of around 115 degrees.

3334-Pos Board B439

Imaging Fluorescence Cross-Correlation Spectroscopy as a Tool to Study Cell-Membrane Organization

Jagadish Sankaran, Nirmalya Bag, Thorsten Wohland.

The structure of biological membranes has been investigated for many years. However, progress is hindered by the fact that putative domains are highly dynamic and their size is smaller than the optical diffraction limit and thus direct observations are difficult. Therefore, there is a need to develop new biophysical tools which can infer the existence of domains within membranes and can follow their development over time. We have introduced in the past a method called Imaging Total Internal Reflection-Fluorescence Correlation Spectroscopy (ITIR-FCS) using EMCCD or sCMOS cameras. ITIR-FCS allows the measurement of a large number (up to ~0.5 million) correlation curves at contiguous locations on cell membranes of live cells with millisecond time resolution. The spatial information within the data can be used to obtain information on the structure and organization of the membranes. This is achieved by calculating differences between the forward and backward cross-correlations between adjacent pixels A and B (CCF_{AB} - CCF_{BA}) or A, B, and C (CCF_{AB} - CCF_{CB}). The results can be depicted as histograms referred to as ΔCCF distributions. In this work we conduct measurements on supported lipid bilayers and cell membranes and perform simulations to demonstrate how ΔCCF distributions change characteristically with membrane complexity and structure. In particular, we demonstrate that domains with sizes below the diffraction limit have a characteristic broadening effect on the ΔCCF distributions. As an example we show that changes in membrane structure and organization of live neuroblastoma cells can be followed over the time course of an hour or more by way of ΔCCF distributions. To deal with large amount of data collected we developed an open source software, ImFCS, to calculate and fit the auto- and cross-correlation functions and depict the results in an imaging format.

3335-Pos Board B440

Probing Orientational Order of MHC Class I Protein and Lipids in Cell Membranes by Fluorescence Polarization-Resolved Microscopy Imaging

Alla Kress, Hubert Ranchon, Patrick Ferrand, Hervé Rigneault, Sophie Brasselet, Tomasz Trombik, Hai-Tao He, Didier Marguet.

Biomolecular orientational organization of lipids and proteins in plasma membrane is a crucial factor in biological processes where functions can be closely related to orientation and ordering mechanisms. The concept of transient nanosized phase separations in ordered and disordered domains, called ''lipid rafts'' is now widely accepted. Furthermore, the ordered domains are enriched in signalling proteins, which highlights the crucial impact of phase separation during the signalling processes. While this field has been so far largely addressed by studying the translational diffusion behaviour of membrane proteins by Single Molecule Tracking or Fluorescence Correlation Spectroscopy, only little is known about the orientational behaviour of signalling proteins in plasma membranes, mainly due to the lack of appropriate rigid fluorescent label which would be able to act as a proper orientation reporter. In this work we develop a fully polarization-resolved fluorescence imaging technique using a tuneable incident polarization state (''fluorescence polarimetry''), in combination with fluorescence anisotropy imaging, in order to provide orientational order information in very general cell membranes shapes.

We apply this technique to the measurement of quantitative orientational distribution of MHC Class I proteins in the plasma and nuclear membranes, benefiting from a rigidly attached GFP probe. The surrounding lipid orientational order in the plasma membrane is additionally probed using the fluorescent reporter di-8-ANEPPQ.The MHC Class I protein is found to be more ordered in the plasma membrane as compared to the nuclear membrane. Both MHC I and di-8-ANEPPQ orientational orders in the plasma membrane are furthermore seen to be highly affected by actin depolymerisation upon Latrunculin A treatment, with variations that indicate both a structural change in the membrane morphology and a disruption of MHC I - actin interactions.

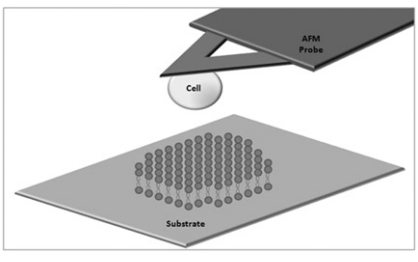
3336-Pos Board B441

A Combined Confocal-Total Internal Reflection Fluorescence (TIRF) Single-Cell Microscopy Investigation of CEACAM1 Dynamics

Laura N. Poloni, Christopher M. Yip.

The carcinoembryonic antigen-related cellular adhesion molecules (CEACAMs) represent a subset of the immunoglobulin superfamily of cell adhesion molecules that mediate intercellular adhesion. One member of this subset, CEACAM1, is particularly interesting since it is down-regulated in tumourigenic cell lines. CEACAM1 exists as an equilibrium between monomers and dimers; however, the spatial and temporal distribution upon cell-substrate contact is not known.

In order to determine CEACAM1 dynamics, a coupled confocal-total internal reflection fluorescence (TIRF)-atomic force microscopy (AFM) platform was used. Live YFP-CEACAM1 labelled cells were isolated on AFM tips for controlled positioning of cells. Confocal microscopy was used to map CEACAM1 receptors over the entire cell surface. Coupled TIRF microscopy monitored the free cell surface as it was brought into contact with a substrate (i.e. another cell, a model membrane, modified glass). Confocal and TIRF microscopy homoFRET measurements were used to determine the distribution of monomeric and dimeric CEACAM1 receptors prior to and upon cell-substrate contact. Through this understanding of how molecular organization affects intercellular binding and signal transduction, it may be possible to identify peptide or pharmacological drug strategies to create CEA CAM-focused therapies for cancer.



3337-Pos Board B442

Following Actin Fibers in 3D During Cell Migration in Collagen Matrices

Michelle A. Digman, Chi-Li Chiu, Jose S. Aguilar, Enrico Gratton.

Actin polymerization is a major mechanism for the production of the force necessary for cell migration in 2D. The polymerization of actin and its retrograde motion at the leading edge of cell moving in 2D has been studied in great detail as well as the interaction of actin with focal adhesions. When cells grow in 3D collagen matrices, the extending lamellipodial protrusion is more difficult to visualize and it is likely not relavent for the movement of the cell over large distances. We use the modulation tracking 3D method to accurately image the cell protrusion. This method is capable of producing detailed images of 3D structures at the nanoscale and at the same time measure diffusion and aggregation of molecules in these structures. In 3D, cells produce very long protrusions that presumably grab on the surrounding collagen fibers to propel the rest of the cell